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Publication date:
2009

Document version
Publisher's PDF, also known as Version of record

Under The High Patronage of His Majesty King Mohammed VI

16th Congress Marrakesh 2009

WVPA

XVIth World Veterinary Poultry Association Congress

Marrakesh, Morocco, November 8-12, 2009

Book of abstracts

ASSOCIATION MAROCAINE DE PATHOLOGIE AVIAIRE

50th Anniversary

Correlation between sequence aided identification and specific PCR tests reported for serovars and related taxa of *Riemerella anatipestifer* investigated by phylogenetic relationships of partial rpoB and 16S rRNA gene sequences

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Diagnosis of infections caused by *R. anatipestifer* (Segers et al. 1993. Int. J. Syst. Bacteriol. 43, 768-776) depends on isolation and identification on the bacterium and are based on traditional biochemical characterization of isolates eventually combined with serotyping. Diagnostic tests based on the detection of antibodies are of limited value since cross reaction with other taxa may happen. Unfortunately unambiguous phenotypical tests for identification of *R. anatipestifer* are lacking and identification based on serotyping and biochemical and physiological characterization therefore might lead to wrong conclusions. The aim of the present investigation was to characterize and identify serovars of *Riemerella anatipestifer* and Riemerella-like isolates genetically and to test the specificity of PCR-tests reported for identification of *R. anatipestifer*. A total of 50 isolates from poultry tentatively classified with *Riemerella anatipestifer* were characterized genetically by partial sequencing of rpoB and by sequencing of the 16S rRNA gene for selected isolates. The results obtained were compared with the data from 13 reference strains by phylogenetic analysis. Fourty-one isolates were identified as *R. anatipestifer*, three as *Wautersiella falsenii-like*, a single isolate obtained from a pigeon as Pelistega europaea while five isolates were classified as new, unnamed taxa. No relationship existed between the 16S rRNA and rpoB gene sequences serovars of *R. anatipestifer*. For example two isolates from ducks previously classified as *R. anatipestifer* serovar 4 and a single isolate from turkeys all of which demonstrate phenotypical characters related with *R. anatipestifer* demonstrated only 75.1% rpoB sequence similarity with *R. anatipestifer* sensu stricto and were later by 16S rRNA sequence comparison found related to the type strain of Wautersiella falsenii. Two PCR tests available from the literature were tested against representative strains from groups identified by rpoB and 16S rRNA gene sequence based comparison. Eight strains identified as *R. anatipestifer* by sequence comparison were positive in both tests. One strain that belonged to *R. anatipestifer* based on sequence comparison, however, only resulted in a weak fragment. Unfortunately bacteria not identified as *R. anatipestifer* according to sequence based indentification also reacted positive in both PCR tests, demonstrating that neither of the tests were specific for *R. anatipestifer*. The present study demonstrated that characterization of *R. anatipestifer* and related bacteria is often inconclusive by traditional methods due to inconsistent reactions and phenotypic diversity and that genotyping at the DNA sequence level is essential to allow proper classification and identification as demonstrated. The present investigations demonstrated that isolates of *R. anatipestifer* often are misidentified, and that new serovars should not be accepted unless they have been properly characterized by genetic methods at the DNA sequence level. In addition, we showed that the published PCR test are not specific for this species. Finally, two new taxa were outlined, the final taxonomic positions of which remain to be identified.